Toxicity of Four Systemic Neonicotinoids to Adults of *Anoplophora glabripennis* (Coleoptera: Cerambycidae)

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ABSTRACT As part of the ongoing evaluation of different systemic insecticides for prophylactic treatment of trees, responses of the beetle *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) to different doses of four systemic neonicotinyl insecticides were studied. Adult beetles were provided with twigs or leaves of trees treated with different concentrations of imidacloprid to evaluate the toxicity of the insecticide through ingestion or contact or through both. Adult beetles also were provided with twigs of host plant treated with clothianidin, dinotefuran, and thiamethoxam to establish dose response of the beetle to these insecticides. Levels of individual insecticides in twigs and leaves were determined by using the "parent" method with high-performance liquid chromatography, and these levels were compared with the applied concentrations to determine their relationship. The LC_{50} values for detected level of each insecticide in twigs was 5.1 ppm at 24 h, 2.9 at 48 h, and 1.9 ppm at 72 h for imidacloprid; 1.1 ppm at 72 h for clothianidin; 2.2 ppm at 72 h for dinotefuran; and 1.0 ppm at 72 h for thiamethoxam. Our results indicate that mortality of adult beetles resulted not only from the ingestion and contact toxicity but also possibly from the antifeedant effect of imidacloprid.

KEY WORDS imidacloprid, dinotefuran, thiamethoxam, clothianidin, LC_{50}

THE CERAMBYCID BEETLE *Anoplophora glabripennis* (Motschulsky) not only caused serious damage to host trees such as poplar (*Populus* spp.) in northern China (Li and Wu 1993, Luo and Li 1999) but also has threatened urban and forest trees, and forestry-related industries in the United States since its discovery in 1996 in New York (Reed 1998). Adult beetles feed on twigs, petioles, and occasionally leaves of host trees. Females chew egg pits in the bark on trunks and large limbs and then lay individual eggs. Hatching larvae feed in the vascular cambium and phloem, and late instars feed in the xylem area (Wang et al. 2000). Damage caused by these feeding behaviors includes disruption of nutrient production and transportation; scarring of twigs, limbs, and trunks; and tunnels in the xylem, which structurally weaken the tree. Infested trees may show early abscission of leaves, die-back of limbs, thinning crowns, and breakage when subjected to stress.

In the United States, tree species that have been found infested include maple (*Acer* spp.), birch (*Betula* spp.), poplar (*Populus* spp.), elm (*Ulmus* spp.), willow (*Salix* spp.), *Aesculus*, and *Platanus*. Additional species, for example, *Elaeagnus augustifolia* L., have been found to be suitable hosts for the beetle in China. Currently, three areas in the United States have programs to eradicate this beetle: Chicago, IL, New York (including Long Island); and northeastern New Jersey (Jersey City and Carteret). An additional infestation in Toronto, Canada, is under eradication by the Canadian Food Inspection Agency. Widespread establishment of the beetle in the United States would potentially result in up to hundreds of billions of dollars in damage to urban areas alone (Novak et al. 2001). The United States program has adopted the use of imidacloprid to treat host trees within a quarter mile of known infested tree(s) to protect them from attack. Reported here is a part of the information that leads to the use of imidacloprid by the program. Among the systemic insecticides that have been evaluated, the neonicotinyl systemic insecticides have been shown to be very promising. Imidacloprid has been shown to be active on insect pests in Homoptera, Coleoptera, Diptera, and Lepidoptera (Mullins 1993) and has been registered in 56 countries for foliar and soil application as well as seed treatment (Leicht 1996). Commercial products with imidacloprid as the active ingredient such as Admire[1], Confidor, Gaucho, Merit, and Premier have been tested and used for controlling insects on field crops and fruit and ornamental trees in both Europe and North America (Altmann and Elbert 1992, Bullock and Pelosi 1994, Stansly et al. 1998). Other recently developed neonicotinyl insecticides such as

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dinotefuran, thiamethoxam, and clothianidin also have been shown to be effective against different insects (Delgarde and Rouland-Lefevre 2002, Corbel et al. 2004, Nault et al. 2004, Wilde et al. 2004). However, little was known about the dose responses of *A. glabripennis* to these chemicals. We have evaluated these insecticides for their efficacy in the field for controlling *A. glabripennis*, and the results will be reported separately. Reported here are the results of laboratory tests to determine the dose–response relationship and hence the concentration (dose) of these insecticides that is required to kill 50% (LC_{50}) and 90% (LC_{90}) of adults.

Materials and Methods

Insecticides. Technical grade of imidacloprid was provided by Bayer CropScience (Kansas City, MO). Imicide, a 4-ml pressurized dispenser, containing either 10 or 15% imidacloprid was provided by J. J. Mauget Co. (Acadia, CA). Technical grade dinotefuran was provided by Valent U.S.A. Corporation (Walnut Creek, CA). We diluted the dinotefuran insecticide with propylene carbonate (99.7% HPLC grade) purchased from Sigma (St. Louis, MO). Technical grade thiamethoxam was provided by Syngenta Crop Protection (Greensboro, NC). J. J. Mauget Co. provided technical grade clothianidin, which was originally obtained from Arvesta Corporation (San Francisco, CA). Clothianidin was diluted using 99.9% methanol (spectrophotometric grade) from Sigma.

Host Plants. Poplar, *Populus deltoides* Bartr. and *Populus nigra* L., and silver maple, *Acer saccharinum* L., were planted in an experimental plot belonging to the Chinese Academy of Forestry, Beijing, China.

Beetles. All live beetles for the test were collected from poplar (*Populus* spp.) or willow (*Salix* spp.) trees that had not been treated with insecticides. Only beetles that seemed healthy were used in the test. Normally, beetles were field collected and used for testing within 2 d. Beetles were challenged by placing them on a flat surface and letting them climb up a cylinder (diameter, 6 cm; height, 20 cm) made of Styrofoam. Only those beetles that successfully climbed at least 15 cm in height were chosen. Beetles were randomly assigned to different treatments.

Test Procedures for Imidacloprid. Two series of tests were conducted with imidacloprid to establish dose response of *A. glabripennis* adults. The first series was done in 2000 and the second in 2001. The first series included the following: 1) total toxicity, i.e., toxicity through contact as well as oral ingestion; 2) ingestion toxicity, i.e., toxicity through oral ingestion only; and 3) contact toxicity, i.e., toxicity through contact only. Respectively, we will refer to these as total toxicity, ingestion toxicity, and contact toxicity test.

Total Toxicity (Dip-Twig). Technical grade of imidacloprid was diluted using distilled water as solvent. Based on the results of a preliminary test, eight water concentrations (21.4, 25, 30, 37.5, 50, 75, 100, and 150 ppm) as well as a control (distilled water) were used to establish dose response of *A. glabripennis* adults to imidacloprid. Freshly cut twigs from silver maple trees were dipped into one of the eight imidacloprid concentrations or water for 3 min, and then they were left to dry at room temperature. The bottom 2 cm of each twig was wrapped with cotton and soaked in water to provide the twig with moisture and then placed individually in a 500-ml jar (upper diameter, ≈7.0 cm; bottom diameter, ≈9.5 cm; height, ≈10.0 cm) in a cylindrical cage (diameter, ≈9.5 cm; height, ≈30.0 cm). Fifteen paired (one male and one female) adults were tested for each concentration. Each pair of adults was introduced into an individual cage with two twigs. Observations were made once every 24 h for a maximum of 6 d, and the status of the beetles was recorded. The area and thickness of twigs consumed by adult beetles were measured and were used together with the residue analysis to determine the total volume of twigs and the amount of insecticide consumed. Twigs were weighed before they were provided to beetles for feeding and were weighed again after they were dried just before the residue analysis. The actual levels of imidacloprid in twigs were determined using a method similar to Placke and Weber (1993). The minimum level of imidacloprid that can be detected using this method was 0.01 ppm.

Ingestion Toxicity. In this test, we attempted to determine the efficacy of imidacloprid when it was only ingested. Imidacloprid concentrations of 25, 37.5, 75, 100, and 150 ppm were tested by brushing individual insecticide solutions onto the bottom side of silver maple leaves along the mid rib. Distilled water served as a control. Treated leaves were dried at room temperature and then folded along the mid rib so that the treated sides were touching each other. Feeding beetles could not contact the treated surface, except with their mouthparts. The folded leaf was stapled along the outside edge and then placed in a 500-ml jar with a pair (a male and a female) of adult beetles. Levels of imidacloprid in leaves were analyzed after the test using the procedure described above. Ten pairs of adult beetles were tested for each concentration and for the control. Adults were checked once every 24 h for a total of 72 h.

Contact Toxicity. The objective of this test was to evaluate the contact toxicity of imidacloprid to the beetle. We also used concentrations of 25, 37.5, 75, 100, and 150 ppm, and distilled water served as the control. Fresh poplar leaves from *P. deltoids* were used for this test because previous experience indicated that beetles are unlikely to feed on them. Leaves were dipped into an imidacloprid solution for 1 min, and then they were placed flat on a clean surface and covered with a petri dish (25 cm in diameter; 1.5 cm in height), which had a hole in the center. Adult beetles were placed on the treated leaf surface and exposed for 30 min. They were then placed in 500-ml glass jars and provided with fresh twigs of untreated silver maple. Ten pairs of adult beetles were tested for each

Table 1. Doses of imidacloprid injected into trunks of *P. nigra* trees and levels of imidacloprid detected in twigs collected from these trees through the method similar to Placke and Weber (1993)

No. dispenses/ 5-cm dbh, % imidacloprid	Imidacloprid (AI) amt (g)/tree	Level (ppm) detected before provided to beetle	Level (ppm) detected after beetle feeding
1, water only	0.000	ND	ND
0.5, 0.1, 4 ml	0.008	ND	ND
0.5, 1.0, 4 ml	0.080	ND	ND
0.5, 10, 4 ml	0.800	ND	ND
2.5, 10, 4 ml	4.000	0.029 ± 0.002	0.020 ± 0.012
3.5, 15, 4 ml	8.400	0.066 ± 0.003	0.055 ± 0.002

ND, not detected.

concentration, including the control. Adults were checked once everyday at 24 h for mortality up to 72 h.

In the second (cut-twig) series test conducted in 2001, imidacloprid was injected in late April into trunks of poplar (*P. nigra*) trees with 20 ± 1.0 cm diameter at breast height (dbh) by using the Mauget system (imicide). Treatments included in the test are listed in Table 1. Twigs (diameter, 0.9 ± 0.1 cm; length, 10.0 ± 1.0 cm) of treated trees were collected in late July and then provided to a pair (one male and one female) of beetles. Fifteen replicates of paired beetles were placed in individual cages with two twigs. At the same time, twigs were freshly cut from these treated trees and analyzed for imidacloprid levels mentioned above. Areas of twigs consumed by individual beetle pairs were measured once every 24 h for a week after the initial placement of adult beetles with twigs or until the beetles died, which also was recorded.

Test Procedure for Dinotefuran, Thiamethoxam, and Clothianidin. Technical grade of dinotefuran (solvent propylene carbonate), thiamethoxam (solvent water) and clothianidin (solvent methanol) were diluted into five concentrations (1, 10, 100, 500, and 1000 ppm) with distilled water as the control (0 ppm). We followed the same procedure as described in the ingestion toxicity test for imidacloprid.

Statistical Analysis for the LC_{50} Data. SAS GLM procedure (SAS Institute 2000) was used to analyze the relationship between applied does and levels of insecticide detected in plant. Probit analysis (Robertson and Preisler 1992) with Polo Plus (LeOra Software, Berkeley, CA) was used to analyze data and to determine LC_{50} and LC_{90} of the detected levels of imidacloprid for each time period and to compare lethal doses in the total toxicity test in 2000. The procedure was also used to determine LC_{50} and LC_{90} for applied doses of imdacloprid in the 2001 test and for the detected levels of the three neonicotinoids in the 2003 test and to compare their lethal doses to that of imidacloprid. SAS PROBIT procedure (SAS Institute 2000) was used to determine the LC_{50} and LC_{90} values of the detected level of each insecticide in twigs for ingestion toxicity test, but with the applied concentration for contact toxicity test because the level of imidacloprid in leaves was not determined. Areas consumed by individual beetles for 1) treated twigs consumed in the total toxicity test and 2) treated leaves consumed in the ingestion toxicity test were analyzed using SAS GLM procedure with a REGWQ test to determine whether there was any relationship between the area consumed and the concentration of imidacloprid.

Results

Relationship between Applied Doses and Levels of Insecticide Detected in Plant. In the first series test, the detected levels of imidacloprid dipped silver maple twigs and surface-treated *P. deltoides* leaves are summarized in Table 2. Generally, levels of insecticide that were detected were 10–25 times less than the applied doses. The relationship between the detected level (Y) and the applied dose (X) can be described using the following two log linear models: 1) $Y = 2.23\ (\pm 0.18)\ \ln(X) - 4.56\ (\pm 0.72)$ for leaf ($R^2 = 0.91$; $F = 149.79$; df = 1, 14; $P < 0.0001$) and 2) $Y = 2.69\ (\pm 0.18)\ \ln(X) - 6.82\ (\pm 0.46)$ for twig ($R^2 = 0.97$; $F = 538.88$; df = 1, 14; $P < 0.0001$).

In the second series test, the levels of imidacloprid detected in twigs collected from poplar trees treated with trunk injection of imidacloprid were very low for all applied doses both before and after twigs were provided to adult beetles (Table 1). In fact, no imidacloprid was detected in twigs of treated trees for the three lower doses. Therefore, a statistical analysis for the relationship between the detected levels of imidacloprid and applied doses was not performed.

Actual levels of clothianidin, dinotefuran, and thiamethoxam in twigs are summarized in Table 3. The relationship between the detected level (Y) and the

Table 2. Levels (mean ± SD) of imidacloprid detected in twigs of silver maple after they were dipped into imidacloprid solution for 3 min and in leaves of *P. deltoides* after they were brushed with imidacloprid solutions

Applied dose (ppm)	Level (ppm) in twigs	Level (ppm) in leaves
150.0	6.25 ± 0.07	6.85 ± 0.49
100.0	5.65 ± 0.07	5.95 ± 0.07
75.0	5.05 ± 0.21	4.55 ± 0.21
50.0	4.10 ± 0.28	3.75 ± 0.07
37.5	3.15 ± 0.07	3.55 ± 0.21
30.0	2.05 ± 0.07	3.90 ± 0.00
25.0	1.75 ± 0.07	2.35 ± 0.07
21.4	1.25 ± 0.07	2.20 ± 0.14
0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3. Levels (mean ± SD) of thiamethoxam, dinotefuran, and clothianidin detected in twigs after they were dipped into different concentrations of the three neonicotinoids for 3 min

Applied dose (ppm)	Clothianidin	Dinotefuran	Thiamethoxam
1	0.14 ± 0.13	0.02 ± 0.03	0.09 ± 0.06
10	1.30 ± 0.33	0.53 ± 0.19	1.70 ± 0.52
100	8.32 ± 0.46	3.18 ± 0.65	6.58 ± 2.10
500	21.1 ± 2.61	10.46 ± 2.17	12.17 ± 1.75
1000	31.72 ± 3.87	19.41 ± 3.48	33.51 ± 1.74

applied dose (X) for each insecticide can be described using the following linear models: 1) $Y = 0.031$ (± 0.002) $X - 2.533$ (± 0.824) for clothianidin ($R^2 = 0.94$; $F = 356.29$; df = 1, 23; $P < 0.0001$), 2) $Y = 0.019$ (± 0.001) $X - 0.576$ (± 0.469) for dinotefuran ($R^2 = 0.95$; $F = 416.99$; df = 1, 23; $P < 0.0001$), and 3) $Y = 0.031$ (± 0.001) $X - 0.873$ (± 0.736) for thiamethoxam ($R^2 = 0.95$; $F = 442.48$; df = 1, 23; $P < 0.0001$). In general, the detected levels in twigs of treated poplar trees for the three neonicotinoids were much less than the applied doses.

Total Toxicity of Imidacloprid in the First Series (Dip-Twig) Test. For all three times (i.e., 24, 48, and 72 h), results of goodness-of-fit tests of the PROBIT model (with normal distribution) indicated an adequate fit of the model ($\chi^2 = 5.36$, df = 6, $P = 0.50$ for 24 h; $\chi^2 = 2.01$, df = 6, $P = 0.92$ for 48 h; and $\chi^2 = 8.43$, df = 6, $P = 0.21$ for 72 h). The slopes (mean ± SE) for the three models are 1.66 ± 0.39 ($t = 4.27$) for 24 h, 1.73 ± 0.43 ($t = 4.03$) for 48 h, and 2.26 ± 0.48 ($t = 4.72$) for 72 h. The hypothesis of equality (equal slopes and equal intercepts) was rejected at $\alpha = 0.05$ ($\chi^2 = 34.82$, df = 4, $P = 0.000$), whereas the hypothesis of parallelism (equal slopes) was not rejected ($\chi^2 = 1.07$, df = 2, $P = 0.59$). The lethal dose ratios (versus 24 h, with 95% CI in parentheses) of LC_{50} and LC_{90} were 1.734 (1.153, 2.610) and 1.868 (0.497, 7.022) at 48 h and 2.608 (1.697, 4.008) and 4.187 (1.364, 12.852) at 72 h, which means that the longer the exposure, the higher the mortality of adult beetles (Fig. 1). The differences among the three dose–response curves were somewhat narrower at lower concentrations than at higher concentrations, indicating a steep increase in the dosage will be necessary when immediate control of the beetle is required. LC_{50} value of imidacloprid detected for adult beetles dropped from 5.1 ppm at 24 h to 1.9 ppm at 72 h (Table 4) when the level detected in twigs was used as the basis of calculation. Adult beetles consumed significantly ($F = 6.34$; df = 8, 261; $P < 0.0001$) less bark and cambium of treated twigs than that of untreated twigs (Table 5). This may be the result of either direct efficacy or possibly an antifeedant effect of imidacloprid on the beetle (Nauen et al. 1998).

The average dry weight of the bark in proportion to surface area for the tested maple twigs was 0.024 g/cm^2. Areas consumed by individual beetles were used to calculate the total amount of imidacloprid ingested by each beetle. Theoretically, LD_{50} based on the amount of imidacloprid ingested can be determined by running a logistic regression of mortality against imidacloprid ingested. However, adult mortality resulted not only from consumption of imidacloprid but also from the direct contact with the insecticide. In addition, the cessation of feeding (Table 5) by beetles exposed to imidacloprid also may have contributed to mortality.

Total Toxicity of Imidacloprid in the Second Series (Cut-Twig) Test. No treatments resulted in >50% adult mortality within 5-d exposure to twigs cut from treated trees. Higher than 50% mortality was achieved only for beetles exposed for 6 d to twigs from trees treated with the two highest doses. This was probably because of the very low levels of imidacloprid found in trunk-injected trees (Table 1). Because of the nondetectable levels of imidacloprid in twigs of treated trees with the three lower doses, no statistical analysis was performed to obtain an LC_{50} value based on the

Table 4. Average LC_{50} and LC_{90} values (95% CI) for imidacloprid when *A. glabripennis* adults had the opportunity to feed and contact treated host tree twigs directly (total toxicity test) for various times after application

	LC_{50}			LC_{90}		
	24 h	48 h	72 h	24 h	48 h	72 h
Detected level of imidacloprid (ppm)	5.1 (3.9–7.9)	2.9 (2.0–3.8)	1.9 (0.8–2.7)	7.1 (14.7–191.6)	16.0 (9.2–73.6)	7.1 (4.7–29.9)

Table 5. Area (mean square centimeters ± SE) consumed by *A. glabripennis* adults when exposed to different doses of imidacloprid

Plant part	Applied dose (ppm)								
	0	21	25	30	37.5	50	75	100	150
Twig	1.414 ± 0.235a	0.055 ± 0.008b	0.072 ± 0.015b	0.060 ± 0.012b	0.041 ± 0.009b	0.101 ± 0.033b	0.032 ± 0.008b	0.575 ± 0.498b	0.040 ± 0.013b
Leaf	0.768 ± 0.137a	NT	0.124 ± 0.036b	NT	0.212 ± 0.066b	NT	0.122 ± 0.037b	0.074 ± 0.023b	0.072 ± 0.014b

For twig (total toxicity test), daily consumption by individual pairs of beetles when they could feed and directly contact treated host tree twigs; for leaf (ingestion toxicity test), daily consumption by individual adult beetles ($n = 20$) when they were allowed to feed on treated leaves without direct contact with treated surfaces.

Means within each row followed by the same letter are not significantly different based on a REGWQ test with $\alpha = 0.05$ (SAS Institute 2000). NT, not tested.

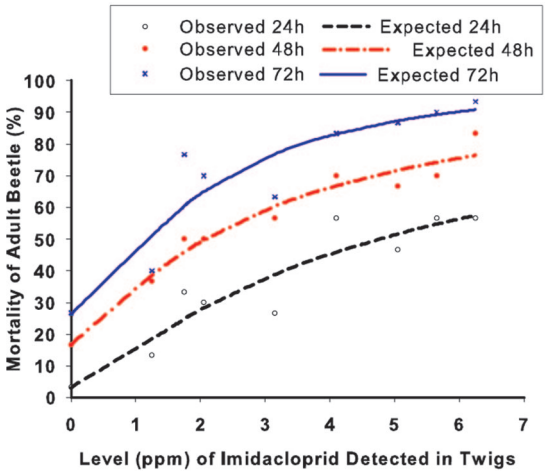

Fig. 1. Mortality of *A. glabripennis* adults feeding and contacting twigs treated with different concentrations of imidacloprid.

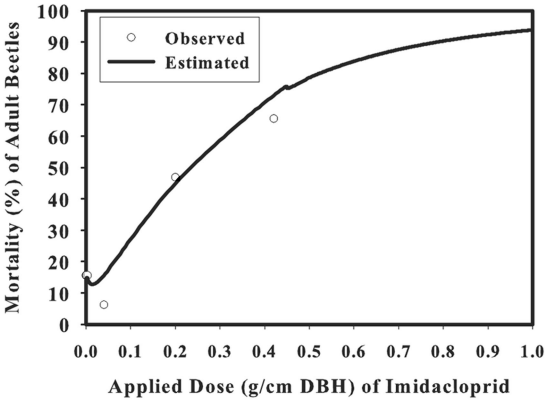

Fig. 2. Mortality of *A. glabripennis* adults when they were provided with twigs collected from trees trunk-injected with different amounts of imidacloprid.

actual level detected in twigs. The analysis was only performed for the relationship between cumulative mortality of adults by the sixth day and the applied dose (grams of imidacloprid per centimeter dbh; Fig. 2). The model fits the data well (Pearson $\chi^2 = 2.638$; df = 3; $P = 0.451$; heterogeneity = 0.879) with the intercept = 1.464 ± 0.472 and the slope = 2.616 ± 0.763 ($t = 3.428$). For poplar trees with 20-cm dbh, the

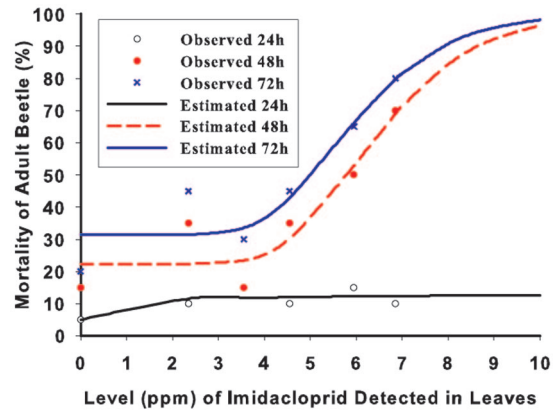

Fig. 3. Mortality of *A. glabripennis* adults feeding on but not directly contacting (other than mouthparts) host tree leaves treated with different imidacloprid concentrations.

LC_{50} at 6 d was 0.276 g/cm dbh with a 95% confidence interval of (0.183–0.418), and the LC_{90} was 0.852 g/cm dbh with a 95% confidence interval of (0.518–4.218).

Ingestion Toxicity for Imidacloprid in the First Series Test. Mortality of adult beetles in all treatments was higher than that in the control. However, there was no clear dose-response effect 24 h after exposure [$\chi^2 = 0.008$, df = 1, $P = 0.929$ for $\log_{10}$(dose)], and mortality was <15% in all treatments (Fig. 3). There was a positive dose–response trend 48 h after exposure, with the intercept = −6.952 ± 3.222 ($\chi^2 = 4.66$, df = 1, $P = 0.031$) and the slope = 8.623 ± 4.010 ($\chi^2 = 4.62$, df = 1, $P = 0.032$). The model fits well (Pearson $\chi^2 = 3.549$, df = 3, $P = 0.315$). The positive dose-response became even clearer at 72 h after exposure, with the intercept = −6.532 ± 3.323 ($\chi^2 = 3.86$, df = 1, $P = 0.049$) and the slope = 8.453 ± 4.4.161 ($\chi^2 = 4.13$, df = 1, $P = 0.042$). The result of goodness-of-fit also indicates that the model fits well (Pearson $\chi^2 = 3.08$, df = 3, $P = 0.380$). Mortality in treatments at 72 h in this test was almost identical to that in the total toxicity test, and it was much higher than it was at 24 h, indicating that ingestion poison played a major role in causing deaths of adult beetles. Again, leaf area consumed by the beetle was much less in treatments than it was in the control. In fact, the higher the applied concentration, the less area of leaves was consumed by adult beetles (Table 5). The LC_{50} values (Table 6) were higher than that in total toxicity test (Table 4) when the beetle not only fed on but also contacted the treated plant parts.

Table 6. Average LC_{50} and LC_{90} values (95% CI) for imidacloprid when *A. glabripennis* adults could feed but could not directly contact treated leaf surfaces (ingestion toxicity test) for various times (hours) after application

	LC_{50}			LC_{90}		
	24 h	48 h	72 h	24 h	48 h	72 h
Detected level of imidacloprid (ppm)	NI	6.4 (4.81–9.67)	5.9 (1.14–7.40)	NI	9.0 (7.38–360.04)	8.4 (6.98–29510.86)

NI, no inference of $LC_{50}$and LC_{90} could be drawn from the data.

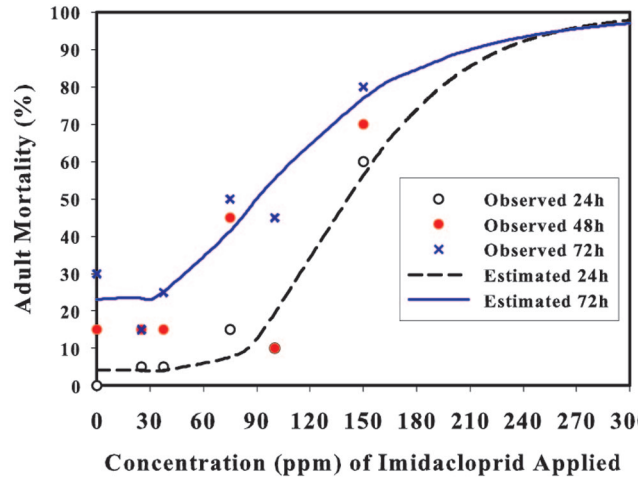

Fig. 4. Mortality of *A. glabripennis* adults contacting, but not feeding on, leaves treated with different concentrations of imidacloprid.

Contact Toxicity for Imidacloprid in the First Series Test. Adult mortality differed in treatments and the control. This difference is marginal within the first 24 h after the treatment, indicating contact poison also contributed to the mortality of the beetles initially (Fig. 4). The intercept for the model is -13.584 ± 7.097 ($\chi^2 = 3.66$, df = 1, $P = 0.056$), and the slope is 6.293 ± 3.333 ($\chi^2 = 2.57$, df = 1, $P = 0.059$). The model fits well (Pearson $\chi^2 = 3.70$, df = 3, $P = 0.295$). The values for the LC_{50} and LC_{90} were 144.1, and 230.2, respectively. Confidence intervals were not estimated for the LC_{50} and LC_{90}. The treatment effect was less obvious 48 h after exposure. Statistical analysis using a SAS Probit procedure did not result in convergence with even 500 iterations. It also yielded a heterogeneity factor H = 3.421 with lack of fit (Pearson $\chi^2 = 10.26$, df = 3, $P = 0.017$). Consequently, a model was not used to fit the data, and a LC_{50} value was not estimated. Only the observed values were plotted (Fig. 4). The higher the doses applied, the higher the mortality (Fig. 4) at 72 h after exposure (Pearson $\chi^2 = 2.93$, df = 3, $P = 0.403$). The intercept for the model is -8.427 ± 3.601 ($\chi^2 = 5.47$, df = 1, $P = 0.019$), and the slope is 4.113 ± 1.740 ($\chi^2 = 5.59$, df = 1, $P = 0.018$). The values for the LC_{50} and LC_{90} were 111.9 with a 95% confidence interval of (61.8, 171.6) and 229.3 with a 95% confidence interval of (157.4, 6616.0), respectively. The increased mortality may result from the

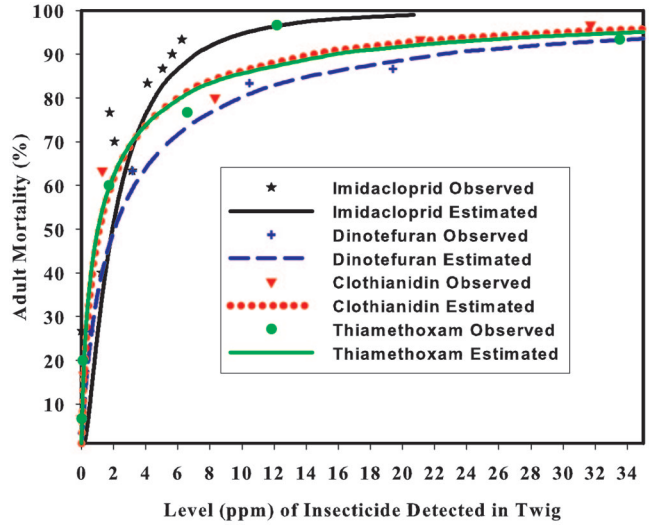

Fig. 5. Mortality of *A. glabripennis* adults feeding and contacting host tree twigs treated with different concentrations of clothianidin, dinotefuran, thiamethoxam, and imidacloprid.

Table 7. Model parameters (mean ± SE, Probit analysis with Polo Plus) of the responses of adults of the Asian long horned beetle to clothianidin, dinotefuran, imidacloprid, and thiamethoxam

Insecticide	C	α	β	Goodness-of-fit test
Clothianidin	0.062 ± 0.042	−0.056 ± 0.180	1.151 ± 0.181 (t=6.36)	χ^2 = 1.61, df = 3, P = 0.657, H = 0.538
Dinotefuran	0.048 ± 0.029	−0.387 ± 0.196	1.234 ± 0.224 (t=5.51)	χ^2 = 0.77, df = 3, P = 0.857, H = 0.258
Imidacloprid	0.263 ± 0.079	−0.643 ± 0.320	2.256 ± 0.504 (t=4.47)	χ^2 = 8.43, df = 6, P = 0.208, H = 1.405
Thiamethoxam	0.070 ± 0.047	−0.011 ± 0.197	1.077 ± 0.199 (t=5.41)	χ^2 = 2.91, df = 3, P = 0.406, H = 0.969

The model can be expressed as $\Pr(\text{response}) = C + (1 - C)F(x'\beta) = C + (1 - C)\ (\Phi\ (\alpha + \beta \log10(\text{dose})))$, where C is a constant, representing natural response, Φ is the normal cumulative distribution function, α is the intercept, and β is the slope; beetles were provided with twigs treated with insecticide solutions for 72 h. H, denotes heterogeneity.

continuing impact of intoxication of the beetle through the contact with treated leaves in the first 30 min.

Total Toxicity of Clothianidin, Dinotefuran, and Thiamethoxam. Generally, dose–response curves of the three insecticides look similar to that of imidacloprid. The estimated value of clothianidin and that of thiamethoxam almost overlaps (Fig. 5). The hypothesis of equality (equal slopes and equal intercepts) is not rejected (χ^2 = 9.42, df = 6, P = 0.151). The hypothesis of parallelism is also not rejected (χ^2 = 5.85, df = 3, P = 0.119). The lethal dose ratios for LC_{50} (versus imidacloprid) at 72 h was 1.704 with a 95% CI of (0.845, 3.436) for clothianidin, 0.888 with a 95% CI of (0.476, 1.656) for dinotefuran, and 1.921 with a 95% CI of (0.892, 4.139) for thiamethoxam, whereas the lethal dose ratios for LC_{90} (versus imidacloprid) at 72 h was 0.490 with a 95% CI of (0.211, 1.138) for clothianidin, 0.315 with a 95% CI of (0.127, 0.779) for dinotefuran, and 0.451 with a 95% CI of (0.185, 1.102) for thiamethoxam. These results indicate that it requires less amount of clothianidin and thiamethoxam, but more dinotefuran than imidacloprid to achieve 50% mortality of adult beetles when they are exposed to these insecticides for 72 h. A different scenario occurs when 90% morality of adult beetles needs to be achieved—more than two-fold of these insecticides compared with imidacloprid. Estimated parameters and the test results are listed in Table 7. The LC_{50} values at 72 h for detected level of each insecticide in twigs are listed in Table 8.

Discussion

The detected levels of the four neonicotinyl insecticides were much lower than the applied doses in this study. The primary reason for this is that the applied doses were represented as ppm of insecticides in solutions, but the detected levels were expressed as ppm of insecticides in dried plant materials. Imidacloprid is supposed to be a systemic insecticide with a high rate of translocation to different parts of plants when it is applied either through soil or trunk injection. Translocation of imidacloprid has been studied mainly for field crops. Gajbhiye et al. (2000) found that imidacloprid was translocated to leaves but not to fruit after seed and root dip treatments and that the translocated residues persisted for 45 d after transplanting tomato plants. Weichel and Nauen (2004) found that the uptake of imidacloprid applied to hop leaves without additives was <10% at 7 d after application. Tattar et al. (1998) reported 1 to 2 ppm imidacloprid detected in foliage of pin oak, *Quercus palustris* Muenchh, 1–12 wk after injecting the insecticide into soil near the root system. However, detected levels were much lower in foliage when imidacloprid was trunk-injected during the 1–13-wk period, especially in the first 4 wk when the concentration was <0.15 ppm. In our second series test, levels of imidacloprid were generally <0.05 ppm even for the two highest doses. One possible explanation may be that compared with smaller trees, larger trees require substantially higher doses of imidacloprid for trunk injection to obtain comparable levels because biomasses increases geometrically with increases in dhb (A. Sawyer and V.C.M., unpublished data). Imidacloprid in smaller (8–10 cm dbh) *P. nigra* trees could reach >5.0 ppm three months after being injected into tree trunks at a rate of one imicide capsule (4 ml 10% imidacloprid) per 5-cm dbh (B.W. et al., unpublished data). Other factors such as tree species, tree health, timing of the application, and weather conditions also may affect to the translocation of imidacloprid in trees. The field samples we collected represent a snapshot of the profile of insecticides in these tree parts. These insecticide levels are dynamic and probably vary considerably form time to time and from one tree part to another. These insecticides can be readily degradable in normal environmental conditions. Imidacloprid, for example, can be rapidly photodegraded into several different chemicals. The photolytic half-life for imidacloprid was 126 min for the formulation Confidor in tap water (Wamhoff and Schneider 1999).

The LC_{50} value at 72 h for *A. glabripennis* adults obtained in this study using the detected level of imidacloprid for any time period was much higher

Table 8. LC_{50} and LC_{90}values of clothianidin, dinotefuran, and thiamethoxam to *A. glabripennis* adults (95% CI)

Insecticide	LC_{50} (ppm)	LC_{90} (ppm)
Clothianidin	1.1 (0.54–1.99)	14.6 (7.90–36.49)
Dinotefuran	2.2 (1.13–3.58)	22.7 (12.08–69.30)
Imidacloprid	1.9 (0.78–4.70)	7.1 (2.73–29.86)
Thiamethoxam	1.0 (0.42–1.84)	14.0 (8.28–44.46)

than that reported for the aphid *Aphis pomi* (De Geer) when the insect was provided with leaf disks treated with imidacloprid for feeding exposure (Lowery and Smirle 2003), indicating a much higher concentration of the insecticide needs to be applied to achieve acceptable management of the beetle. In our contact only test, we placed beetles in a confined small container with imidacloprid. For many volatile insecticides, this would normally cause problems because the insecticide also could act as a fumigant. However, Scholz and Reinhard (1999) reported none or a minimal amount of volatiles based on the complete mass balance in their study on the photolysis of imidacoprid.

The results of our test indicated that the adult beetles of *A. glabripennis* may not only be intoxicated by ingesting imidacloprid but also through contact with treated surfaces. Antifeedant effects (Nauen et al. 1998) also may contribute to the mortality because daily consumption of treated plants reduced with the increase in dosage.

Dose–response curves of adult *A. glabripennis* to clothianidin, dinotefuran, and thiamethoxam were generally similar to that of imidacloprid although the LC_{50} value for both clothianidin and thiamethoxam at 72 h was approximately one-half of that of dinotefuran and imidacloprid. The reasons for the similarities and differences may be that, although all four tested insecticides are neonicotinoids, the binding sites, insecticidal, and neural activities may differ because of their structural differences. Imidacloprid had similar insecticidal potency with clothianidin and dinotefuran when the insecticides were directly injected into adult male American cockroach, *Periplaneta americana* (L.), but caused higher nerve excitatory activity than dinotefuran and clothianidin (Kiriyama and Nishimura 2002). After analyzing the binding of several neonicotinoids to membranes of *Myzus persicae* (Sulzer) and *Aphis craccivora* Koch Kayser et al. (2004) concluded that acetamiprid and clothianidin share the same biding site with imidacloprid, whereas thiamethoxam binds to a different site or in a different mode. Differences in the toxicity of the four insecticides also may result from differential metabolism by insects as well as tree tissues. In our test series one, we only evaluated LC_{50} value for beetles fed on or exposed to insecticides for not >72 h. The longevities of these beetles were likely to be shorter with a higher variance than laboratory reared beetles because they were collected from the field and their ages were unknown. Although LC_{50} values were quite high for all neoticotinoids at 72 h, an areawide program where all host trees are treated should ensure longer exposure and therefore lower the LC_{50} values significantly as the results of our test series 2 indicate.

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